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REPORT ON A  
"STUDY OF THE PLATEAU  
OF  
MICROBIOLOGICAL CONTAMINATION ON SURFACES"

15 April 1967  
Task 5.3  
JPL CONTRACT 951624

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CONTENTS

<u>Section</u>	<u>Page</u>
I. Introduction.....	1
II. Test Plan and Procedures .....	2
III. Results .....	6
IV. Discussion .....	38
V. Conclusions .....	39
VI. Recommendations .....	41
VII. References .....	41

ILLUSTRATIONS

<u>Figure</u>		<u>Page</u>
1.	Die-Off of <u>Staphylococcus epidermidis</u> on Stainless Steel in EASL Laminar Flow and in the Control Environment .....	7
2.	Die-Off of <u>Staphylococcus epidermidis</u> on Aluminum in the EASL Laminar Flow and in the Control Environment .....	8
3.	Die-Off of <u>Staphylococcus epidermidis</u> on Conversion-Coated Magnesium in EASL Laminar Flow and in the Control Environment .....	9
4.	Die-Off of <u>Staphylococcus epidermidis</u> on Conformal Coating in EASL Laminar Flow and in the Control Environment ....	10
5.	Die-Off of <u>E. coli</u> on Stainless Steel in EASL Laminar Flow and in the Control Environment .....	11
6.	Die-Off of <u>E. coli</u> on Aluminum in EASL Laminar Flow and in the Control Environment .....	12
7.	Die-Off of <u>E. coli</u> on Conversion-Coated Magnesium in EASL Laminar Flow and in the Control Environment .....	13
8.	Die-Off of <u>E. coli</u> on Conformal Coating in EASL Laminar Flow and in the Control Environment .....	14
9.	Die-Off of <u>Bacillus globigii</u> on Stainless Steel in EASL Laminar Flow and in the Control Environment .....	15
10.	Die-Off of <u>Bacillus globigii</u> on Aluminum in EASL Laminar Flow and in the Control Environment .....	16
11.	Die-Off of <u>Bacillus globigii</u> on Conversion-Coated Magnesium in EASL Laminar Flow and in the Control Environment .....	17
12.	Die-Off of <u>Bacillus globigii</u> on Conformal Coating in the EASL Laminar Flow and in the Control Environment.....	18
13.	Die-Off of EASL Spore, <u>Bacillus pumilus</u> (M-2) on Stainless Steel in EASL Laminar Flow and in the Control Environment .....	19

ILLUSTRATIONS (Concl'd)

<u>Figure</u>	<u>Page</u>
14. Die-Off of EASL Spore, <u>Bacillus pumilus</u> (M-2) on Aluminum in EASL Laminar Flow and in the Control Environment...	20
15. Die-Off of EASL Spore <u>Bacillus pumilus</u> (M-2) on Conversion Coated Magnesium in EASL Laminar Flow and in the Control Environment .....	21
16. Die-Off of EASL Spore <u>Bacillus pumilus</u> (M-2) on Conformal Coating in EASL Laminar Flow and in the Control Environment .....	22

TABLES

<u>Table</u>		<u>Page</u>
I.	Die-Off of <u>Staphylococcus epidermidis</u> when Exposed to the EASL Laminar Flow Environment .....	23
II.	Die-Off of <u>Staphylococcus epidermidis</u> when Exposed to the EASL Laminar Flow Environment .....	24
III.	Die-Off of <u>Escherichia coli</u> when Exposed to the EASL Laminar Flow Environment.....	25
IV.	Die-Off of <u>Escherichia coli</u> when Exposed to the Control Environment .....	26
V.	Die-Off of <u>Bacillus globigii</u> Spores when Exposed to the EASL Laminar Flow Environment .....	27
VI.	Die-Off of <u>Bacillus globigii</u> Spores when Exposed to the Control Environment .....	28
VII.	Die-Off of <u>Bacillus pumilus</u> (M-2) when Exposed to the EASL Environment .....	29
VIII.	Die-Off of <u>Bacillus pumilus</u> (M-2) when Exposed to the Control Environment .....	30
IX.	Die-Off of a Mixture of EASL Isolates Exposed to the EASL Environment on Steel, Aluminum, Conversion, and Conformal-Coated Surfaces .....	31
X.	Die-Off of a Mixture of EASL Isolates Exposed to the Control Environment on Steel, Aluminum, Conversion, and Conformal-Coated Surfaces .....	32
XI.	The Accumulation of Biological Burden on Fallout Strips of Stainless Steel, Aluminum, Conformal-Coated Material, and Conversion-Coated Magnesium Exposed to the EASL Environment .....	33
XII.	EASL Environment, Temperature, and Humidity During the Course of Task 5.3, Contamination Plateau Study .....	34
XIII.	Die-Off of Microorganisms Exposed to the EASL Laminar Flow Environment .....	36
XIV.	Die-Off of Microorganisms Exposed to the EASL Control Environment .....	37

ABBREVIATIONS

cm <sup>2</sup>	Square centimeter
°C	Degrees centigrade
EASL	Experimental Assembly and Sterilization Laboratory
°F	Degrees fahrenheit
ft	Feet
hrs	hours
in <sup>2</sup>	Square inches
kc	Kilocycle
min	Minute
ml	Milliliter
NVPR	No viable Particles Recovered
RH	Relative humidity
TSA	Trypticase soy agar
WG	Water gage



## I. INTRODUCTION

The purpose of this study was to determine the die-off rate of individual species and mixed flora of microorganisms exposed on strips of selected materials to different environmental conditions (EASL Facility vertical laminar air flow area versus non-laminar flow).

Microorganisms (bacterial vegetative cells and spores) are killed by a variety of mechanisms when exposed to natural environmental conditions such as the ultra violet of sun light, lack of nutrients, desiccation, heat and cold. This study was restricted to examining the effects of desiccation produced by exposure in controlled humidity and temperature ovens and in the laminar air-flow area of the EASL (Bioassay Room) of Building 233 at JPL. Kill by desiccation is usually induced by denaturation of the microbial protein, and/or a possible oxidative process. Microorganisms, if protected by residual culture medium, detritus, dirt, grease, organic matter, dust, or other microorganisms, usually show a greater resistance to kill than unprotected microorganisms. In the EASL area, the flow of air (75 ± 20 ft/min) is greater than that in the oven (almost static). As a result of this air flow there might be an enhancement of the effects of desiccation in the EASL area resulting in a more rapid kill.

## II. TEST PLAN AND PROCEDURES

### A. GENERAL APPROACH

Microorganisms (EASL isolates and others) were exposed to the EASL laminar flow and to the controlled temperature and humidity (oven) environments. Cell suspensions of the microorganisms were dried on strips (or coupons) of representative materials which might be used for spacecraft construction. The strips/coupons were placed in uncovered Petri dishes in the EASL environment. The strips/coupons were elevated in the Petri dishes by laying them on sterile packing foam or other suitable material. The strips/coupons exposed in the controlled environment oven were in covered Petri dishes. At periodic intervals the strips/coupons were removed and cultured aerobically on TSA at 32°C. In this manner, the die-away kinetics of the exposed organisms were determined. A control system of sterile strips was exposed in the EASL environment to assist in determining the possible buildup or plateauing of biological burden which might occur in the EASL Facility.

### B. MICROORGANISMS EXPOSED TO THE EASL AND OVEN ENVIRONMENTS

1. Staphylococcus epidermidis
2. Bacillus globigii (spores)
3. Escherichia coli
4. Spores of an EASL isolate (M-2 which was B. pumilus subspecies B)
5. A mixed flora of EASL isolates. Three non-spore formers and 1 spore former (M-2 B. pumilus subspecies B, E-17 Arthrobacter globiformis, E-6 M. luteus, and E-9 M. rhodochrous).
6. Organisms deposited by exposure to the EASL environments (EASL situation only).

### C. MICROORGANISMS AND PREPARATION OF SUSPENSIONS

1. All vegetative cells were grown aerobically on TSA at 32°C for 18-24 hours.
  - a. The spores were grown on TSA supplemented with M<sub>n</sub> and C<sub>a</sub> salts.
2. The organisms were harvested by washing them off the agar with sterile distilled water or peptone water (1 percent).

3. The organisms were pooled, centrifuged at 0°C, and washed. The harvested vegetative cells were washed three times in cold 1-percent peptone water, and the spores were washed five times in chilled sterile distilled water (0° to 4°C).
4. Plate counts were done to determine the number of viable organisms per unit sample.
5. A final cell suspension was made containing  $10^5$  viable organisms per ml.

#### D. COMPOSITION AND SIZE OF TEST STRIP/COUPONS

1. Coupons were 1 cm<sup>2</sup>.
2. Test strip/coupons made of stainless steel, aluminum, a conformal-coated (casteroil/polyurethane over stainless steel) surface, and conversion-coated magnesium (Dow 7) were used.
3. Strips to determine EASL fall-out were 1 x 3 x 0.02 inches of stainless steel, aluminum, conversion-coated magnesium, and conformal-coated materials.

#### E. DEPOSITION OF TEST ORGANISMS ON THE TEST STRIPS/COUPONS

1. Suspension of the test organism (0.01 ml) was deposited on the sterile coupon by means of a calibrated pipette.
2. The coupons with the organisms were dried at 32°C.
3. After the organisms on the strips were dried, sample strips were withdrawn and the number of viable organisms per coupon was determined by culturing. (See subsections II. H-1 and 2 for culture technique.)

#### F. EXPOSURE OF THE TEST STRIPS IN THE EASL AND CONTROLLED ENVIRONMENTS

1. The test strips exposed in the EASL environment were in Petri dishes without lids, as described in subsection II. A.
2. The test strips in the controlled environment were in Petri plates with lids.

## G. CONTROLS FOR THE STRIPS EXPOSED TO THE EASL ENVIRONMENT

1. Sterile control strips were exposed to the EASL environment and the buildup and/or plateauing of microbial flora was determined.
2. The control strips were to be used as a means of determining the EASL microbial contribution to the strips inoculated with the laboratory organisms, e.g., E. coli or S. epidermidis. This procedure would have been used if it were not possible to distinguish (by colonial morphology or pigmentation) the original laboratory organisms from the EASL contributed flora. This was found to be unnecessary.
3. Five strip/coupons were assayed prior to inoculation to verify sterility of the surfaces to be inoculated.

## H. DETERMINATION OF POPULATION LEVELS OF THE TEST STRIPS AFTER ENVIRONMENTAL EXPOSURE

1. The exposed strips were placed in 1-percent sterile peptone water and sonicated for 12 minutes at 25 kc/sec. Aliquots of the peptone water and the strip were plated on TSA and incubated aerobically at 32°C for 72 hours.
2. The plates were counted after 24, 48, and 72 hours.

## I. EXPOSURE TIMES AFTER THE TEST STRIPS WERE EXPOSED TO BOTH ENVIRONMENTS

1. Petri dishes containing the test strips were exposed to both environments.
2. Five replicates of each organism on four different types of test strips/coupons and the control strips/coupons were exposed and withdrawn from the EASL and the control environments using the following schedule:
  - a. E. coli: 2, 4, 6, 24, and 48 hours.
  - b. S. epidermidis: 6, 24, 48, and 72 hours; 5, 10, and 14 days.
  - c. B. globigii spores: 1, 5, 10, 20, 30, 40, and 50 days.
  - d. A mixed flora of EASL isolates: 1, 5, 10, 20, 30, 40, and 50 days.
  - e. EASL isolate spore (B. pumilus): 1, 5, 10, 20, 30, 40, and 50 days.

- f. Flora deposited by EASL: 6, 24, 48, and 72 hours; 5, 10, 30, 40, and 50 days.

#### J. CONTROLLED ENVIRONMENT

1. A vacuum oven at atmospheric pressure--kept at 25°C--was used to produce the controlled environment.
2. The oven contained a vessel half-filled with an aqueous saturated solution of  $K_2CO_3 \cdot H_2O$ .
3. By maintaining the desiccator with the saturated  $K_2CO_3 \cdot H_2O$  solution at 25°C, a 42.8-percent RH was produced.

#### K. EASL ENVIRONMENT

1. Exposed EASL samples were kept within environmental specifications of the EASL vertical laminar flow bioassay area.
  - a. Temperature:  $(70^\circ \pm 10^\circ F)$
  - b. Relative Humidity:  $(45 \pm 5\text{-percent RH})$
  - c. Velocity:  $(75 \text{ ft/min} \pm 20 \text{ ft/min})$
  - d. Pressure: (a minimum of 0.05 in WG pressure differential overall over pressurized or nonpressurized environments.)
2. The preceding parameters were monitored and records kept.
3. During several days, the EASL facility went off specification. (See Table XII.)

### III. RESULTS

Figures 1-4 are the die-off curves for S. epidermidis on stainless steel, aluminum, conversion-coated magnesium, and conformal-coated surfaces when exposed to the EASL and the control environments. Tables I and II give the numerical values from which Figures 1-4 were drawn.

Figures 5-8 are the die-off curves for E. coli on stainless steel, aluminum, conversion-coated magnesium, and conformal-coated surfaces when exposed to the EASL environment and the control environment. Tables III and IV give the numerical values from which Figures 5-8 were drawn.

Figures 9-12 are the die-off curves for B. globigii spores on stainless steel, aluminum, conversion-coated magnesium, and conformal-coated surfaces when exposed to the EASL environment and the control environment. Tables V and VI give the numerical values from which Figures 9-12 were drawn.

Figures 13-16 are the die-off curves for Bacillus pumilus subspecies B (M-2, an EASL isolate) spores on stainless steel, aluminum, conversion-coated magnesium and conformal-coated surfaces when exposed to the EASL environment and the control environment. Tables VII and VIII give the numerical values from which Figures 13-16 were drawn.

Tables IX and X list the surviving populations from a mixture of the four EASL isolates, M. luteus (E6), M. rhodochrous (E9), A. globiformis (E17), and B. pumilus spores (M2), on stainless steel, aluminum, conversion-coated magnesium, and conformal-coated materials when exposed to the EASL laminar downflow and the control environment.

Table XI shows the microbiological burden obtained from exposing sterile strips of the test materials in the EASL facility for the 50-day duration of the experiment.

Table XII is a record of the temperature and humidity of EASL during the experiment. The facility went off specifications February 2-7, 1967 and February 20-28, 1967, the main problem being the humidity control.

Tables XIII and XIV are summations of all data from the die-off curves for E. coli, S. epidermidis, EASL isolates mixture, B. globigii spores, and B. pumilus spores.

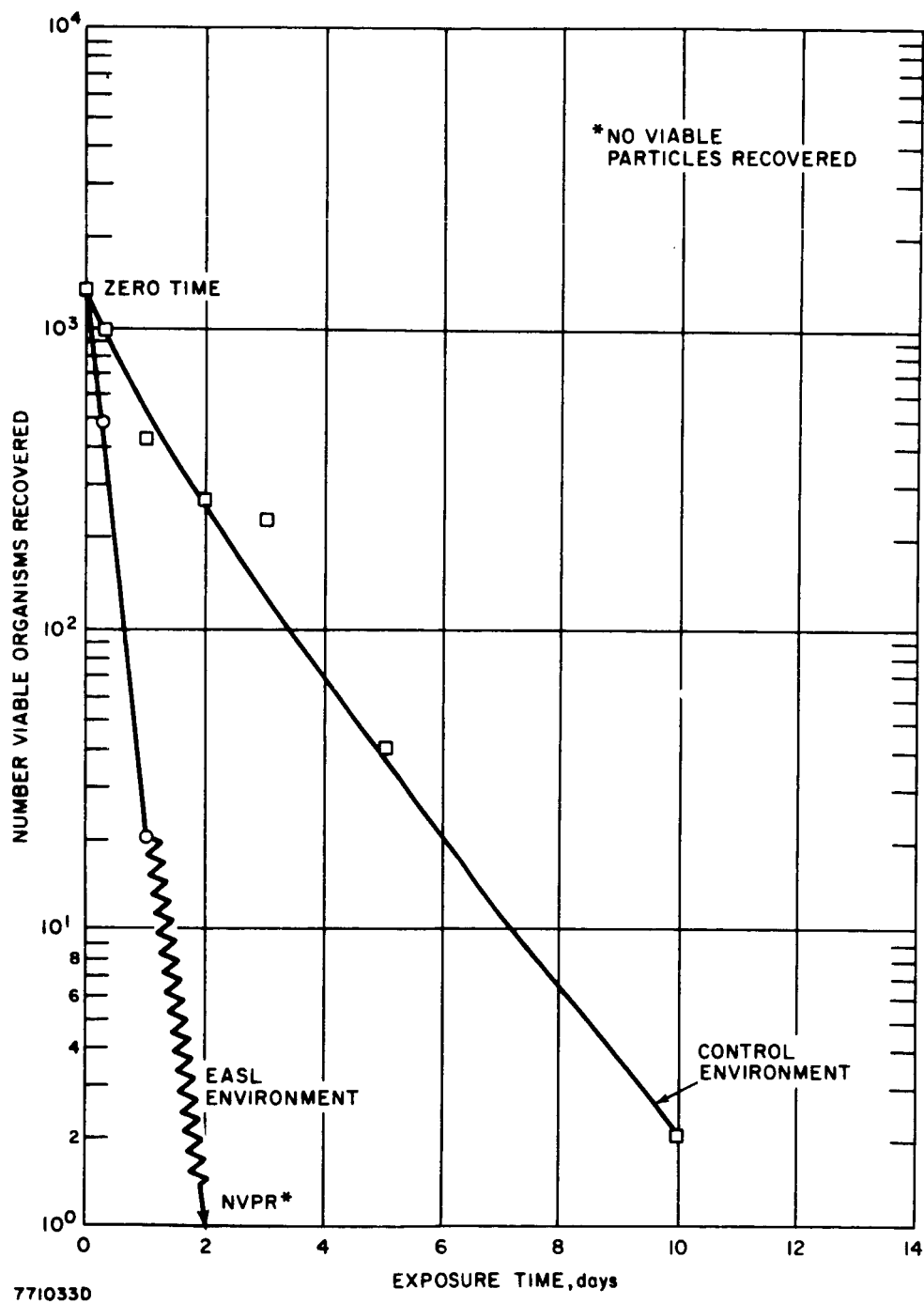
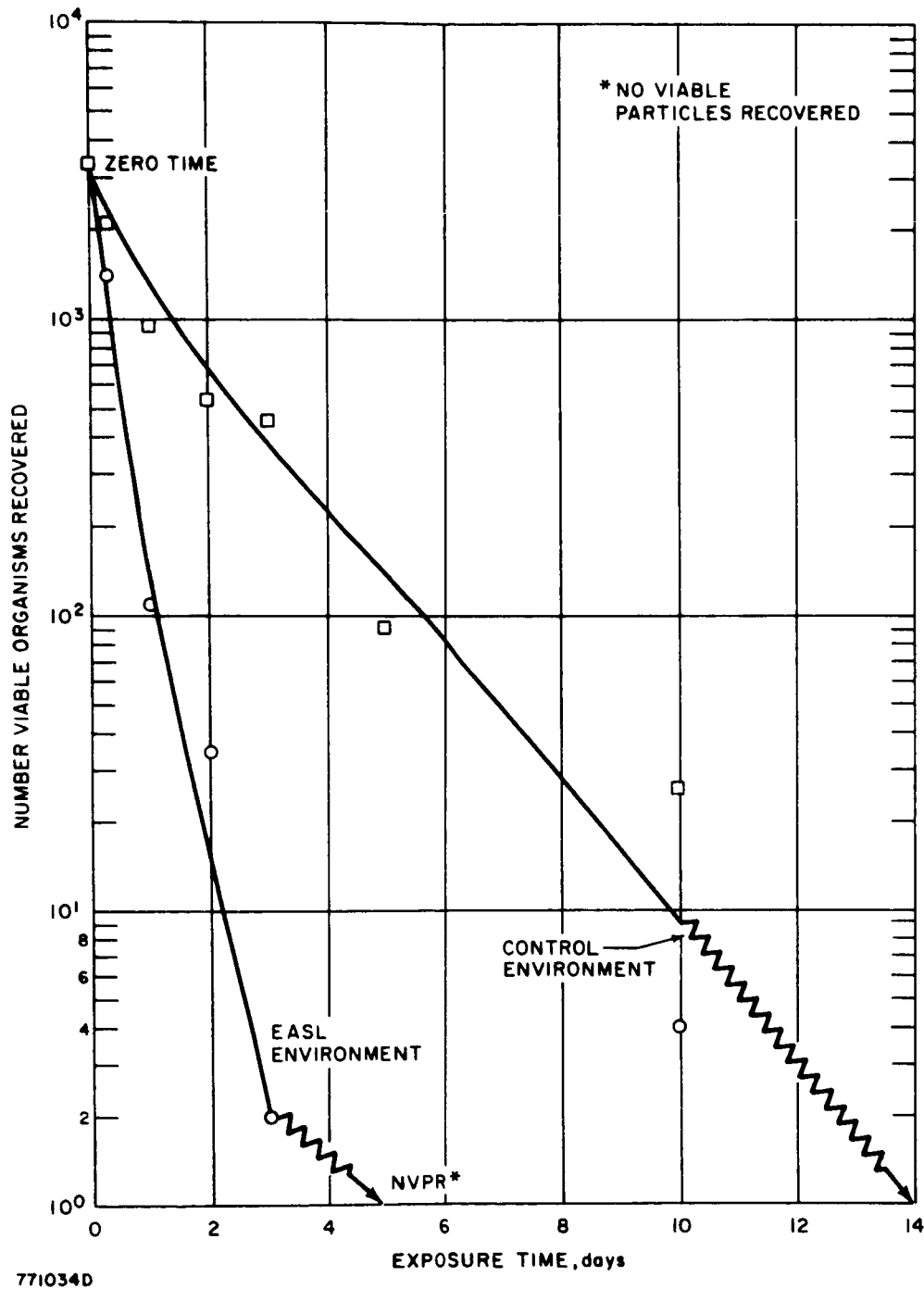


Figure 1. DIE-OFF OF *STAPHYLOCOCCUS EPIDERMIDIS* ON STAINLESS STEEL IN EASL LAMINAR FLOW AND IN THE CONTROL ENVIRONMENT



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Figure 2. DIE-OFF OF *STAPHYLOCOCCUS EPIDERMIDIS* ON ALUMINUM  
IN THE EASL LAMINAR FLOW AND IN THE CONTROL  
ENVIRONMENT



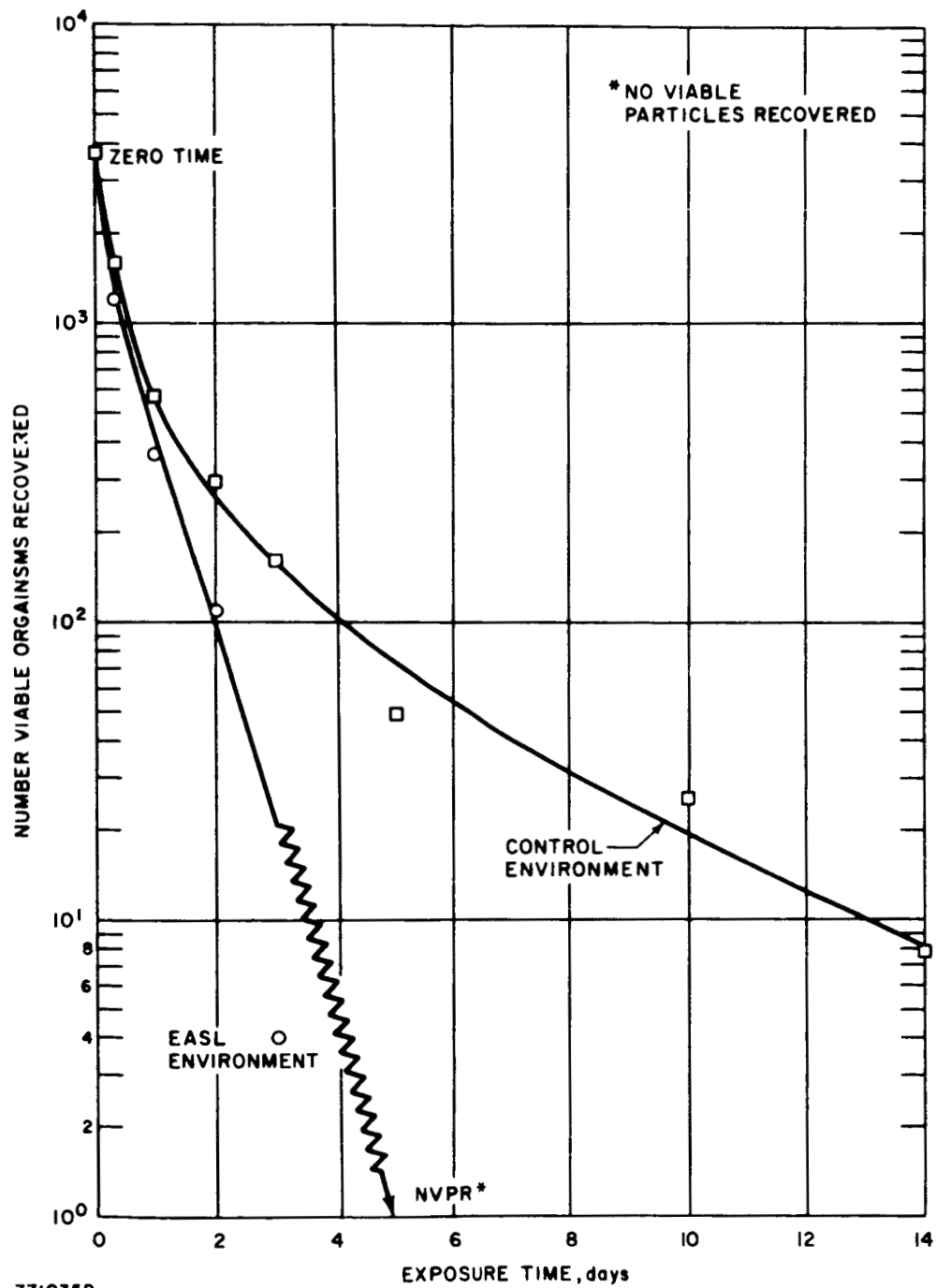


Figure 3. DIE-OFF OF *STAPHYLOCOCCUS EPIDERMIDIS* ON CONVERSION-COATED MAGNESIUM IN EASL LAMINAR FLOW AND IN THE CONTROL ENVIRONMENT

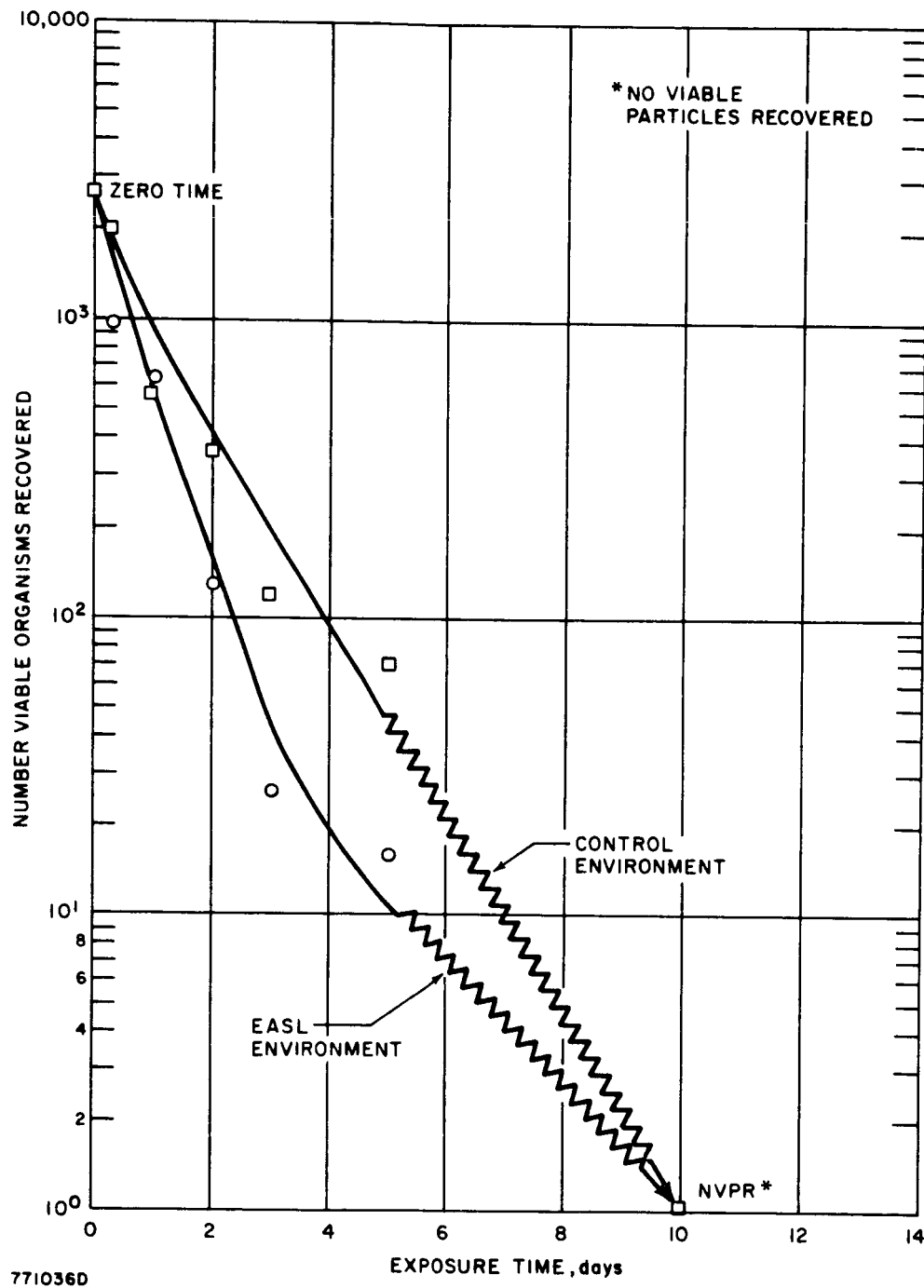


Figure 4. DIE-OFF OF STAPHYLOCOCCUS EPIDERMIDIS ON CONFORMAL COATING IN EASL LAMINAR FLOW AND IN THE CONTROL ENVIRONMENT

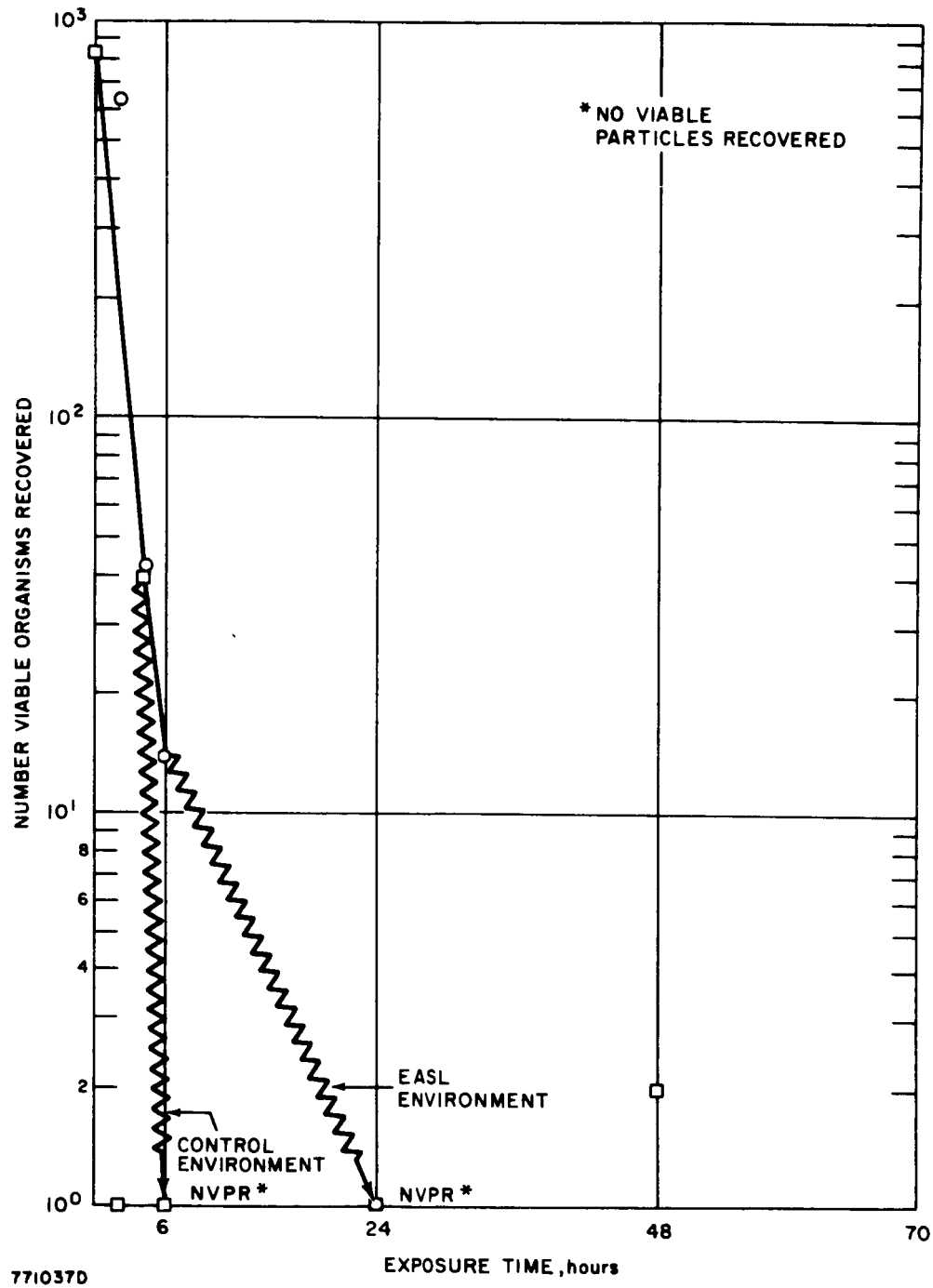


Figure 5. DIE-OFF OF E. COLI ON STAINLESS STEEL IN EASL LAMINAR FLOW AND IN THE CONTROL ENVIRONMENT

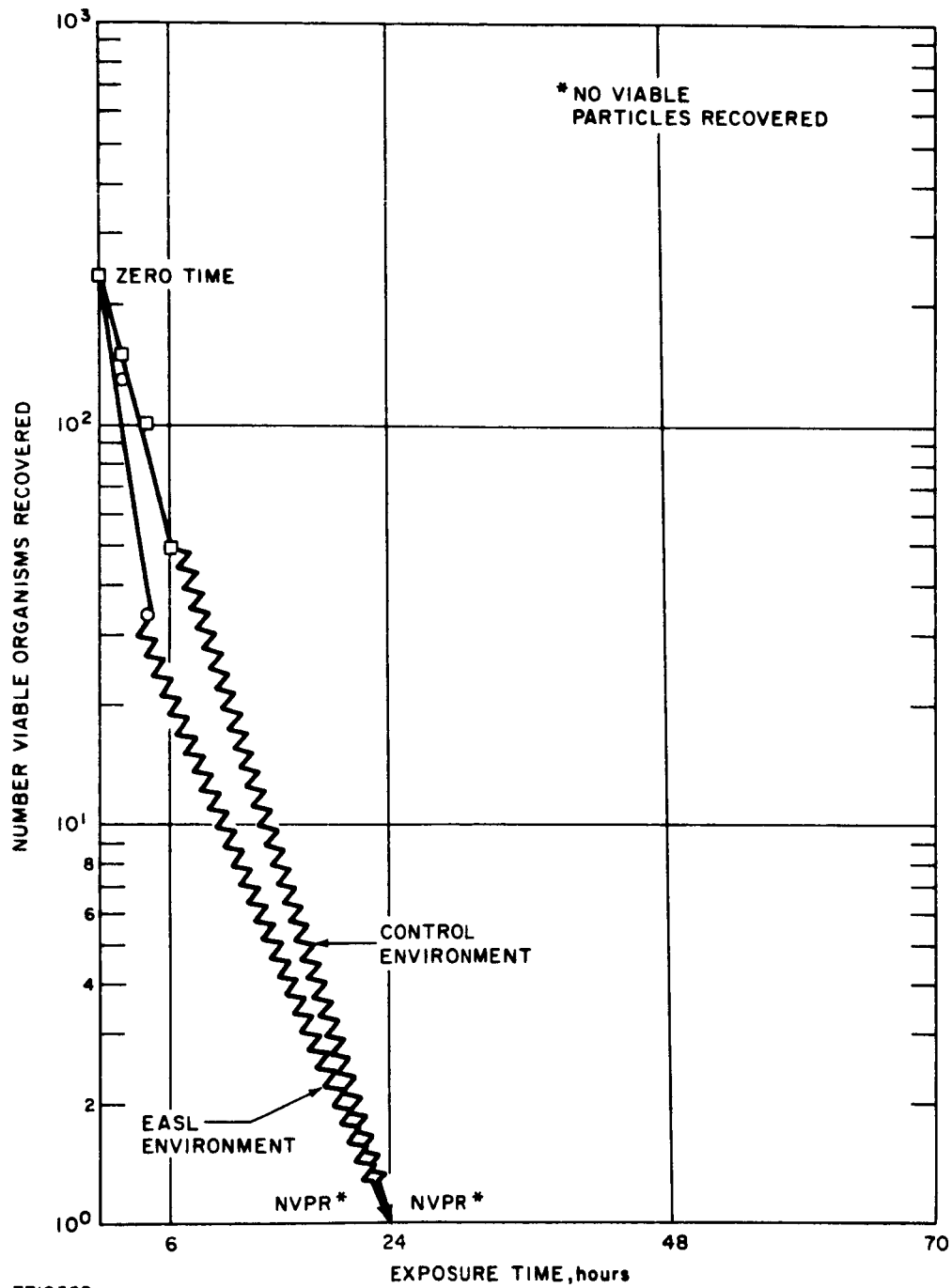


Figure 6. DIE-OFF OF E. COLI ON ALUMINUM IN EASL LAMINAR FLOW AND IN THE CONTROL ENVIRONMENT

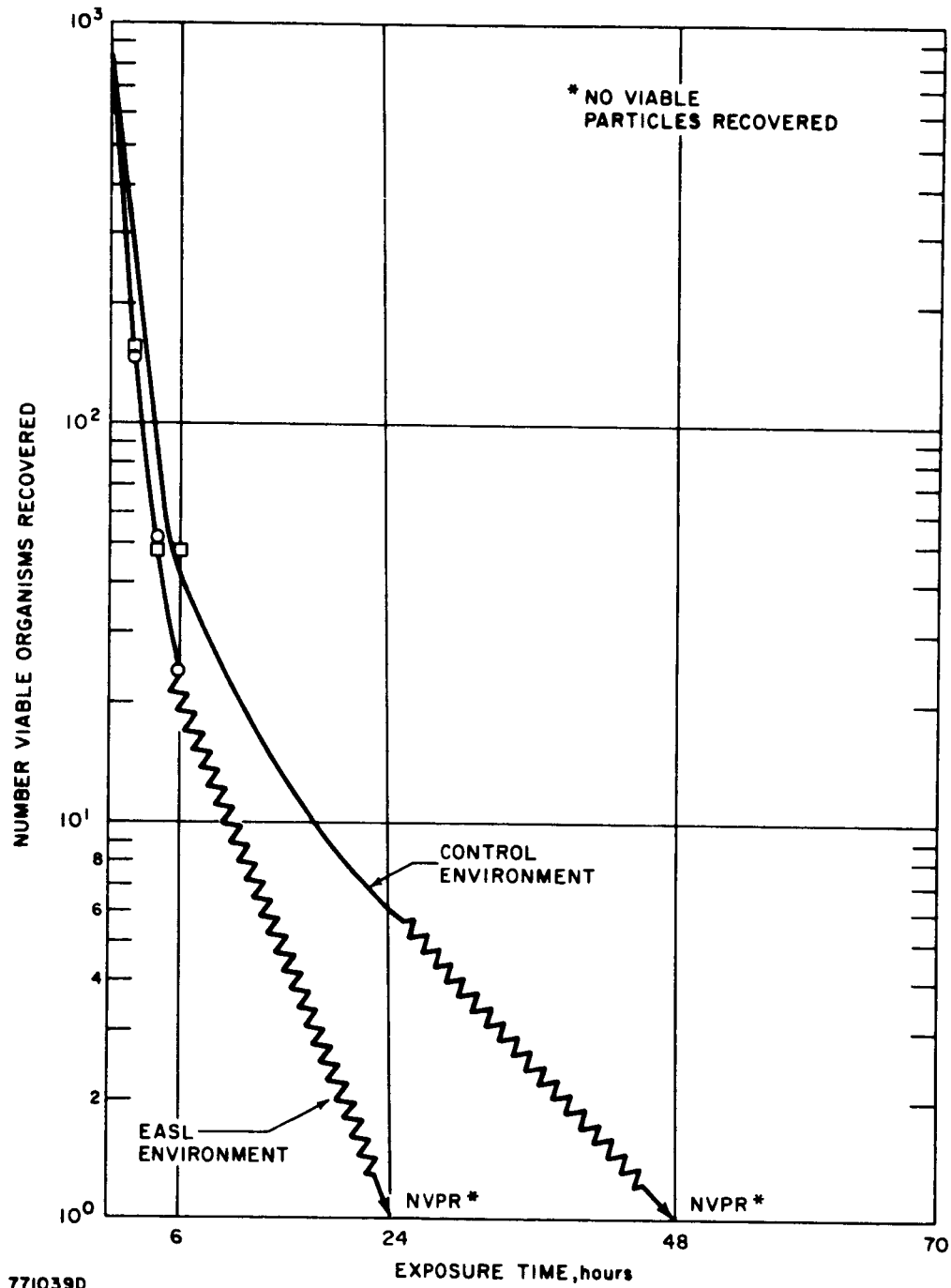


Figure 7. DIE-OFF OF *E. COLI* ON CONVERSION-COATED MAGNESIUM IN EASL LAMINAR FLOW AND IN THE CONTROL ENVIRONMENT

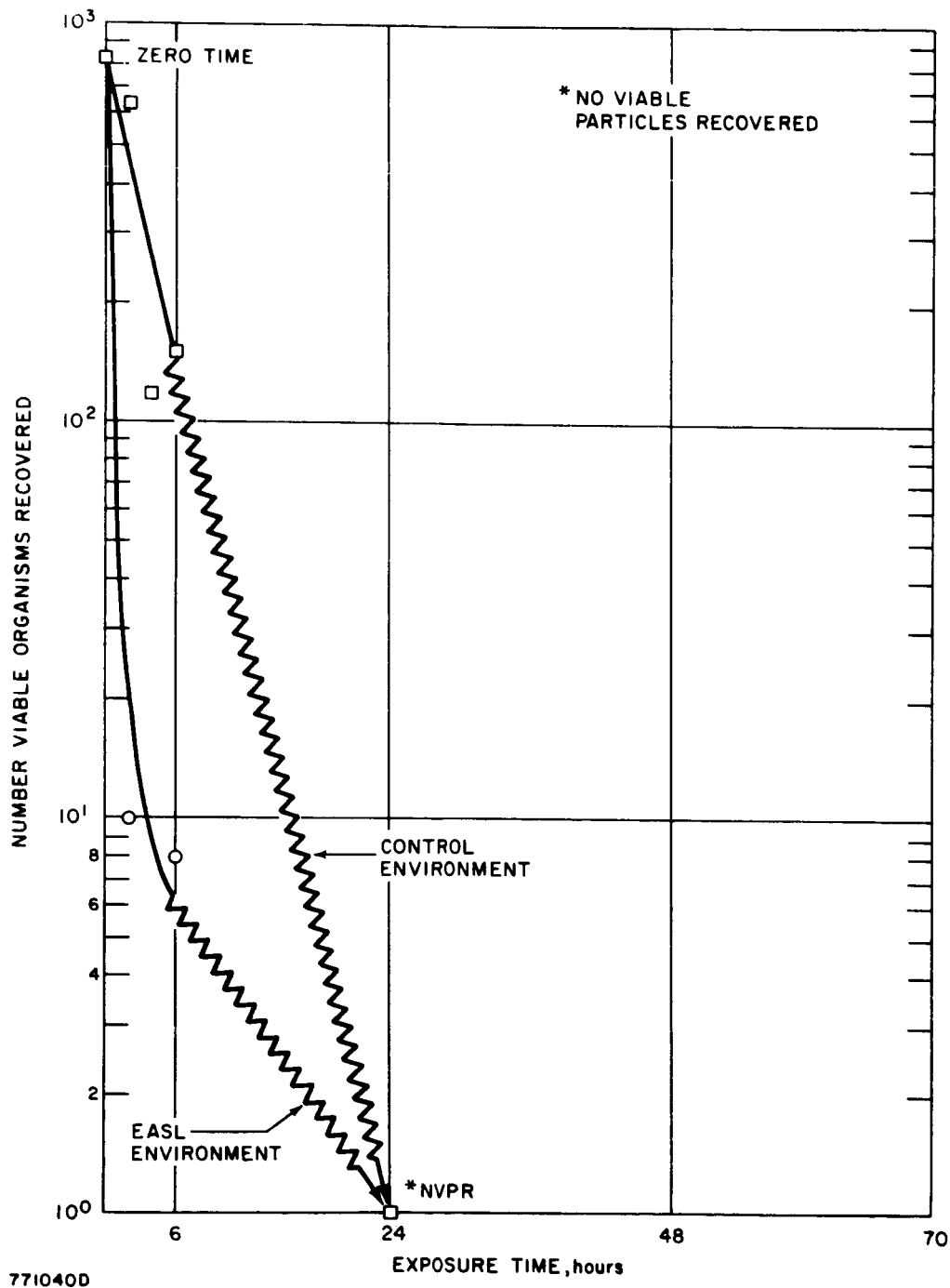


Figure 8. DIE-OFF OF *E. COLI* ON CONFORMAL COATING IN EASL LAMINAR FLOW AND IN THE CONTROL ENVIRONMENT

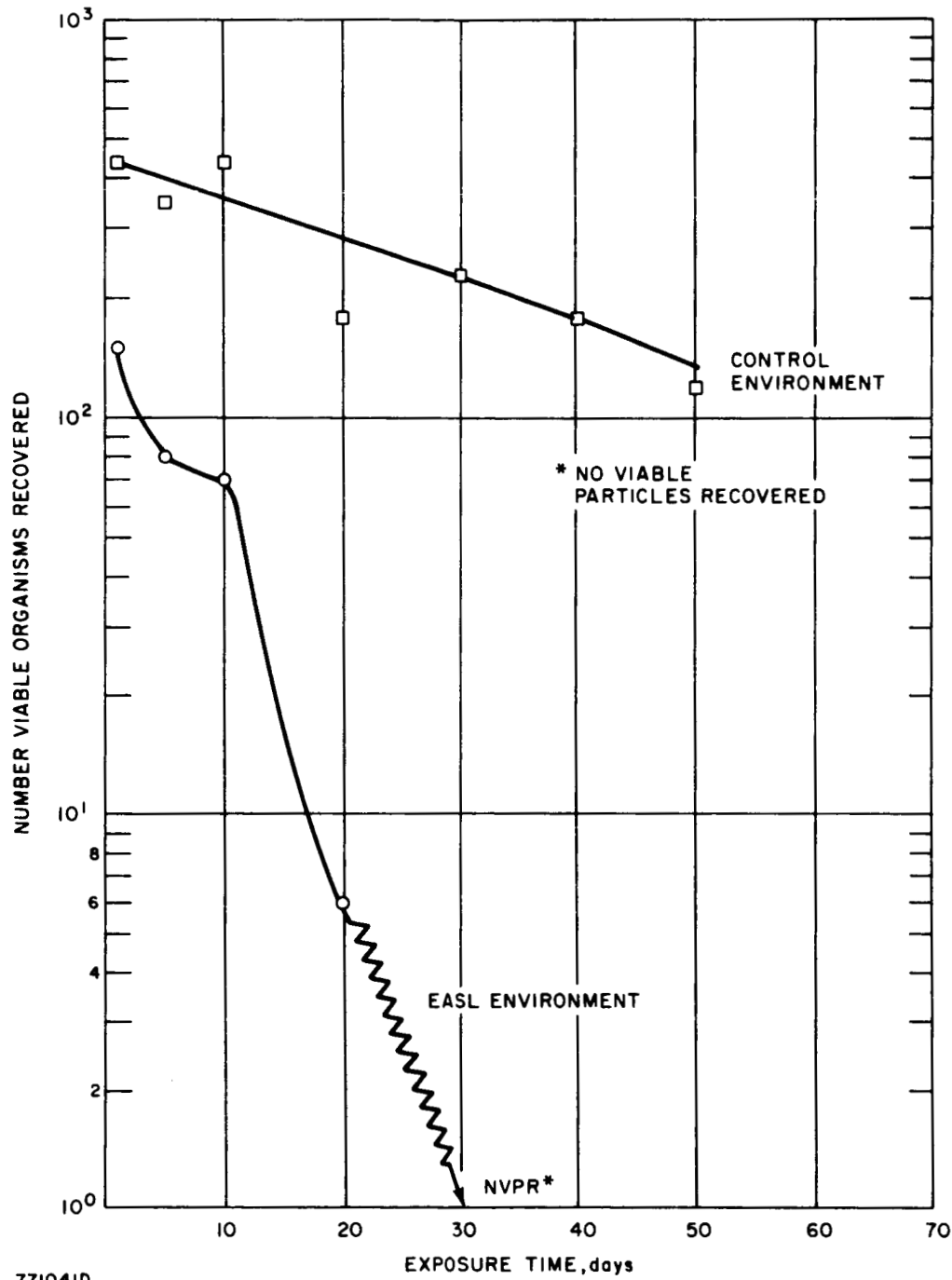


Figure 9. DIE-OFF OF BACILLUS GLOBIGII ON STAINLESS STEEL IN EASL LAMINAR FLOW AND IN THE CONTROL ENVIRONMENT

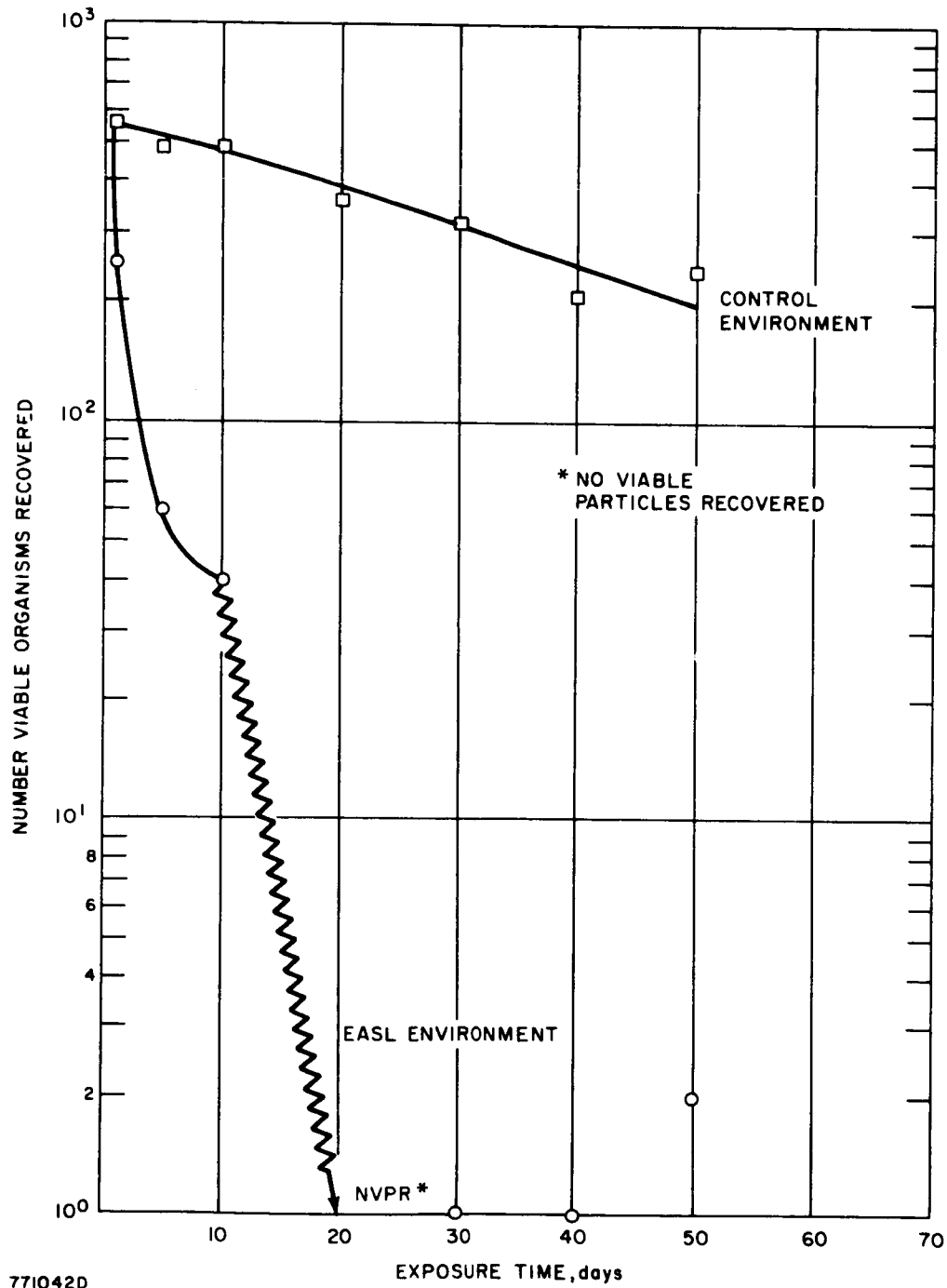


Figure 10. DIE-OFF OF *BACILLUS GLOBIGII* ON ALUMINUM IN EASL LAMINAR FLOW AND IN THE CONTROL ENVIRONMENT



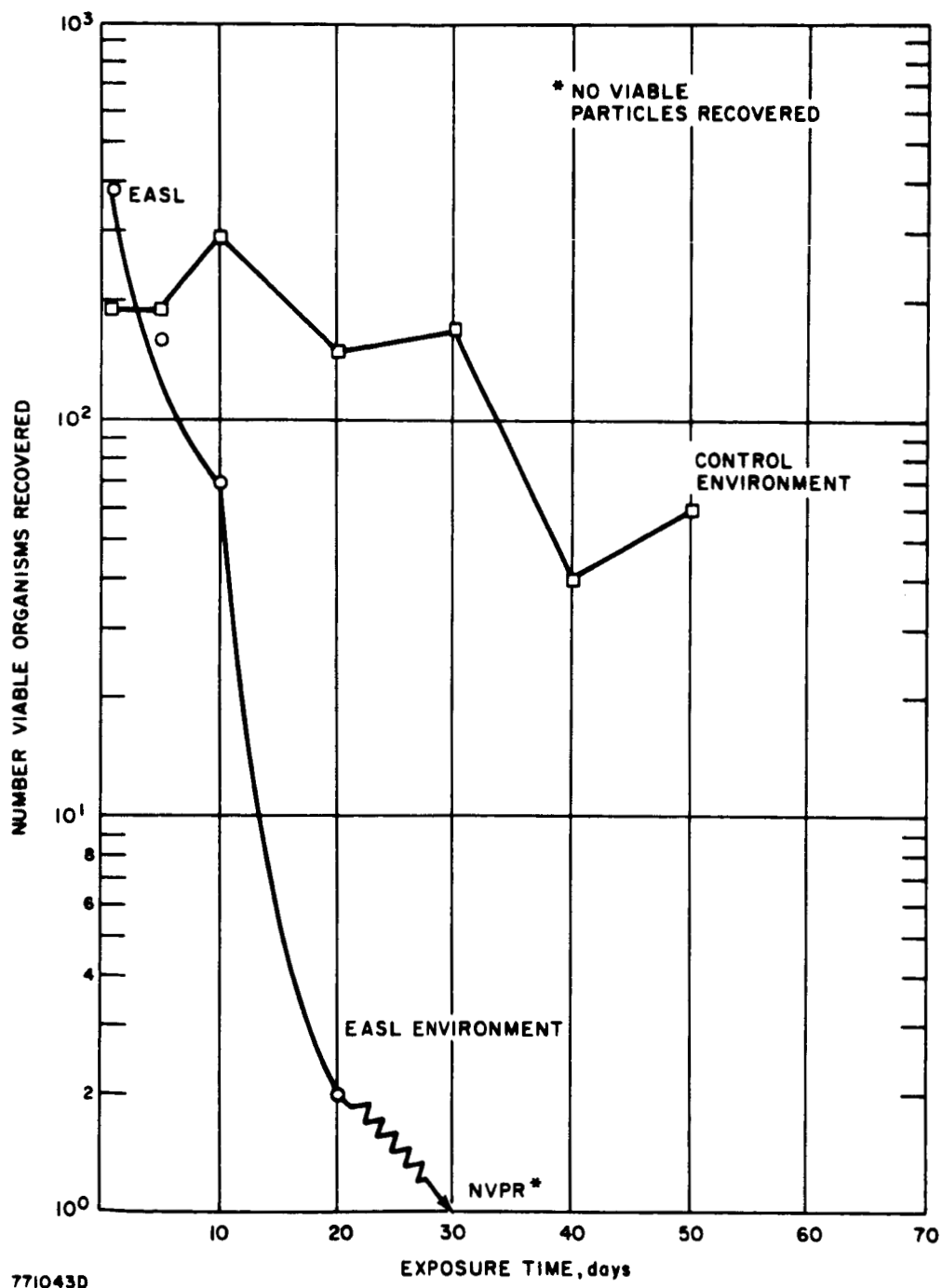


Figure 11. DIE-OFF OF *BACILLUS GLOBIGII* ON CONVERSION-COATED MAGNESIUM IN EASL LAMINAR FLOW AND IN THE CONTROL ENVIRONMENT

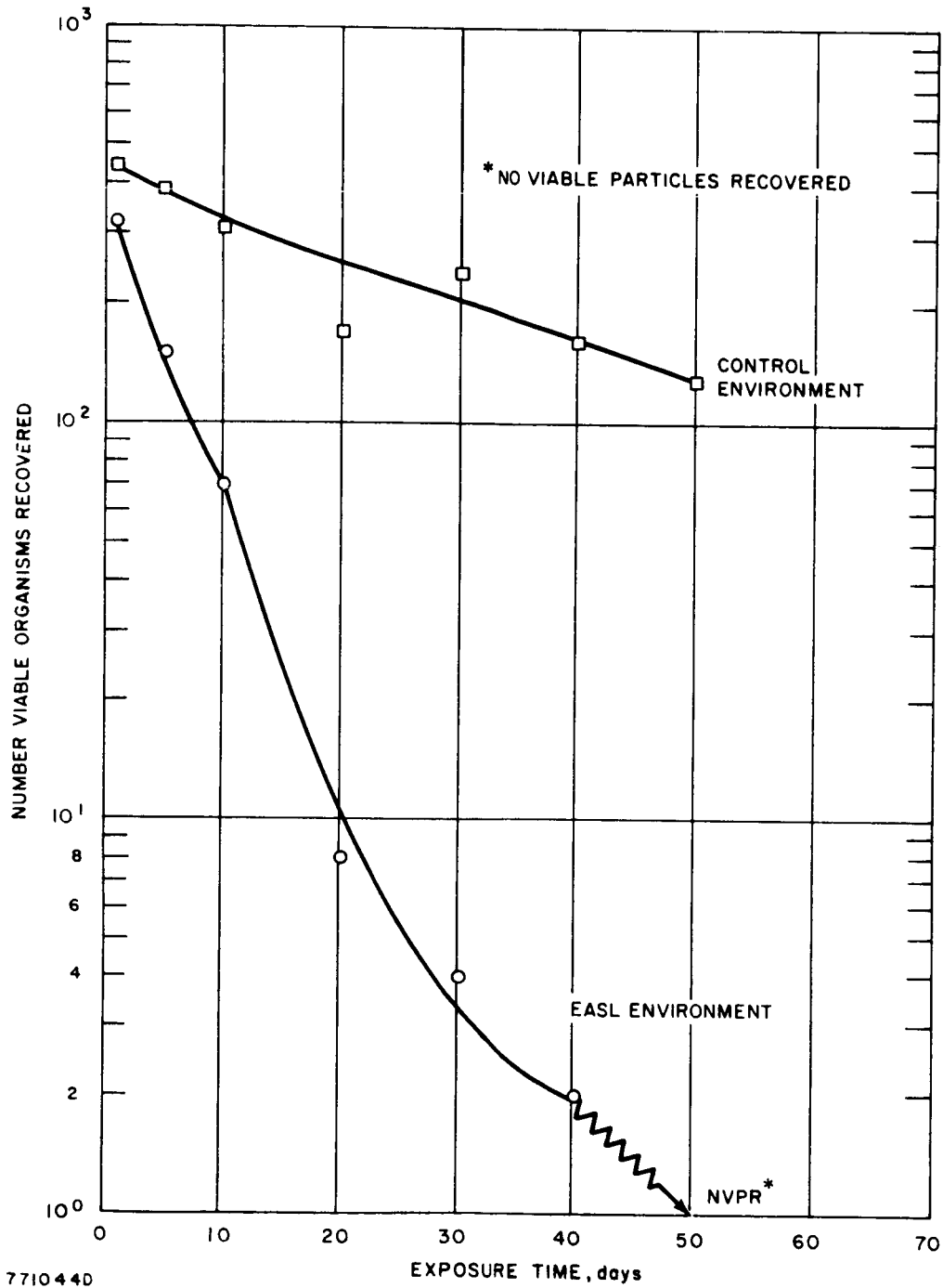


Figure 12. DIE-OFF OF BACILLUS GLOBIGII ON CONFORMAL COATING  
IN THE EASL LAMINAR FLOW AND IN THE CONTROL ENVIRONMENT

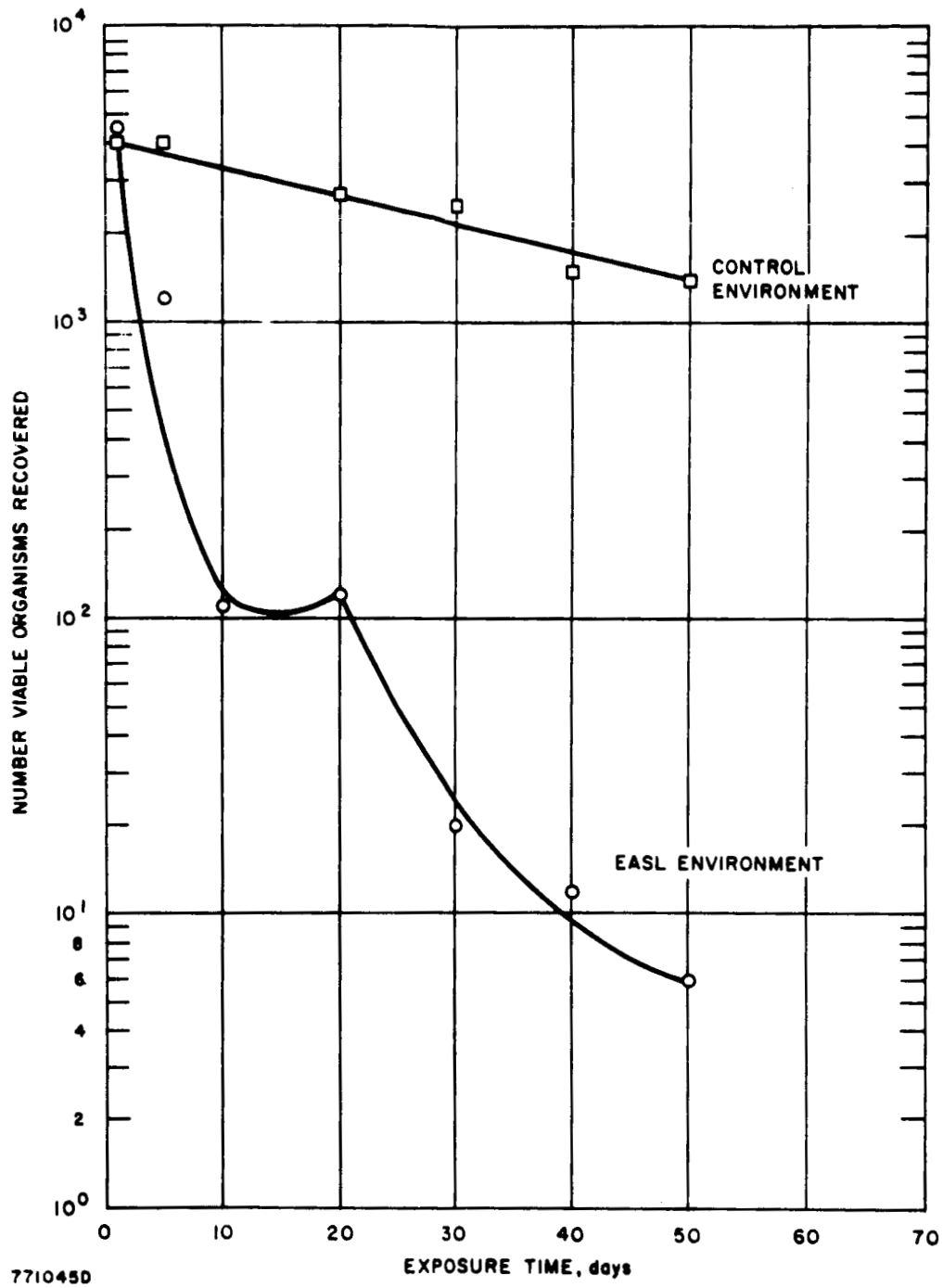


Figure 13. DIE-OFF OF EASL SPORE, *BACILLUS PUMILUS* (M-2) ON STAIN-LESS STEEL IN EASL LAMINAR FLOW AND IN THE CONTROL ENVIRONMENT

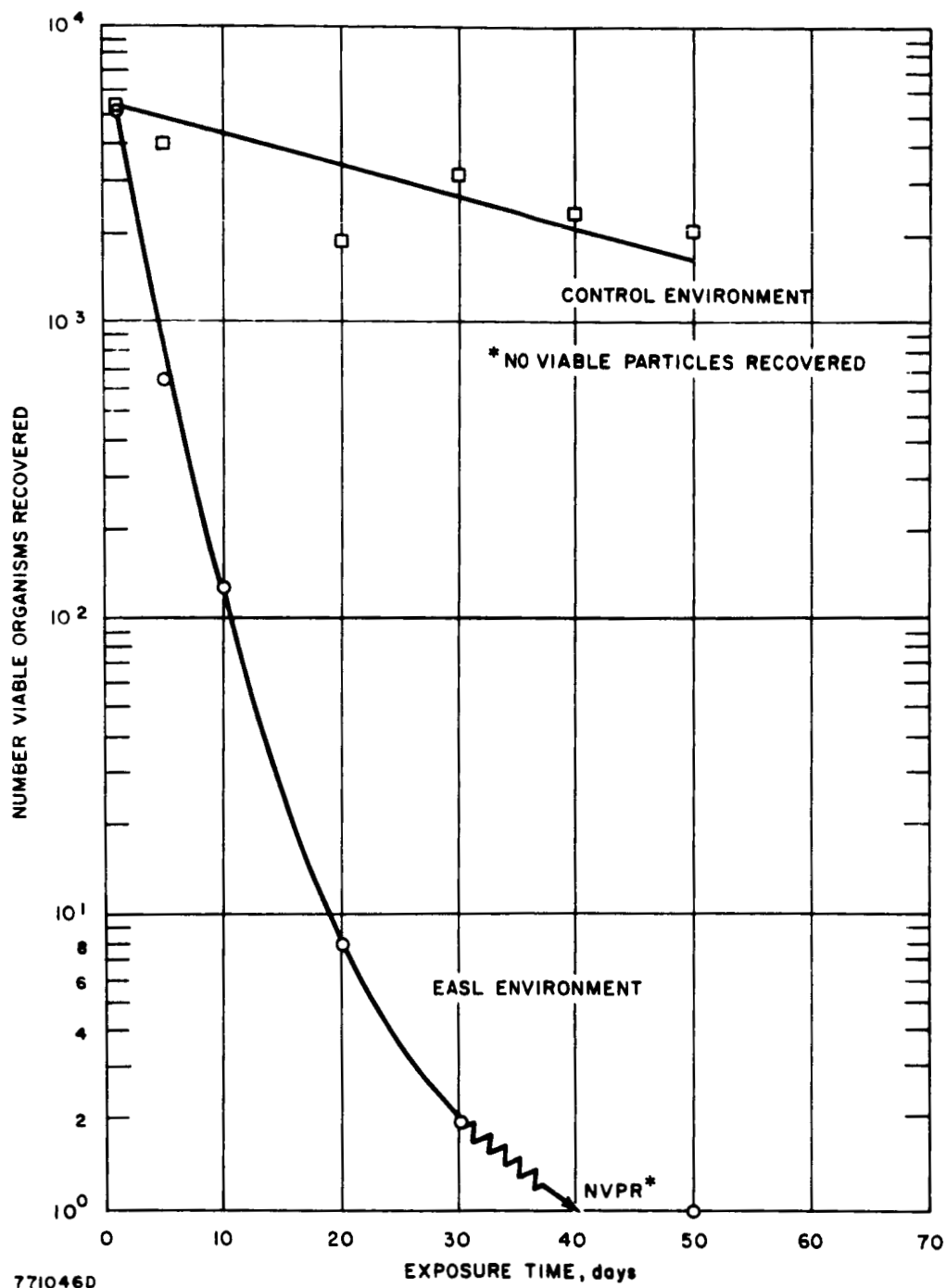


Figure 14. DIE-OFF OF EASL SPORE, BACILLUS PUMILUS (M-2) ON ALUMINUM IN EASL LAMINAR FLOW AND IN THE CONTROL ENVIRONMENT

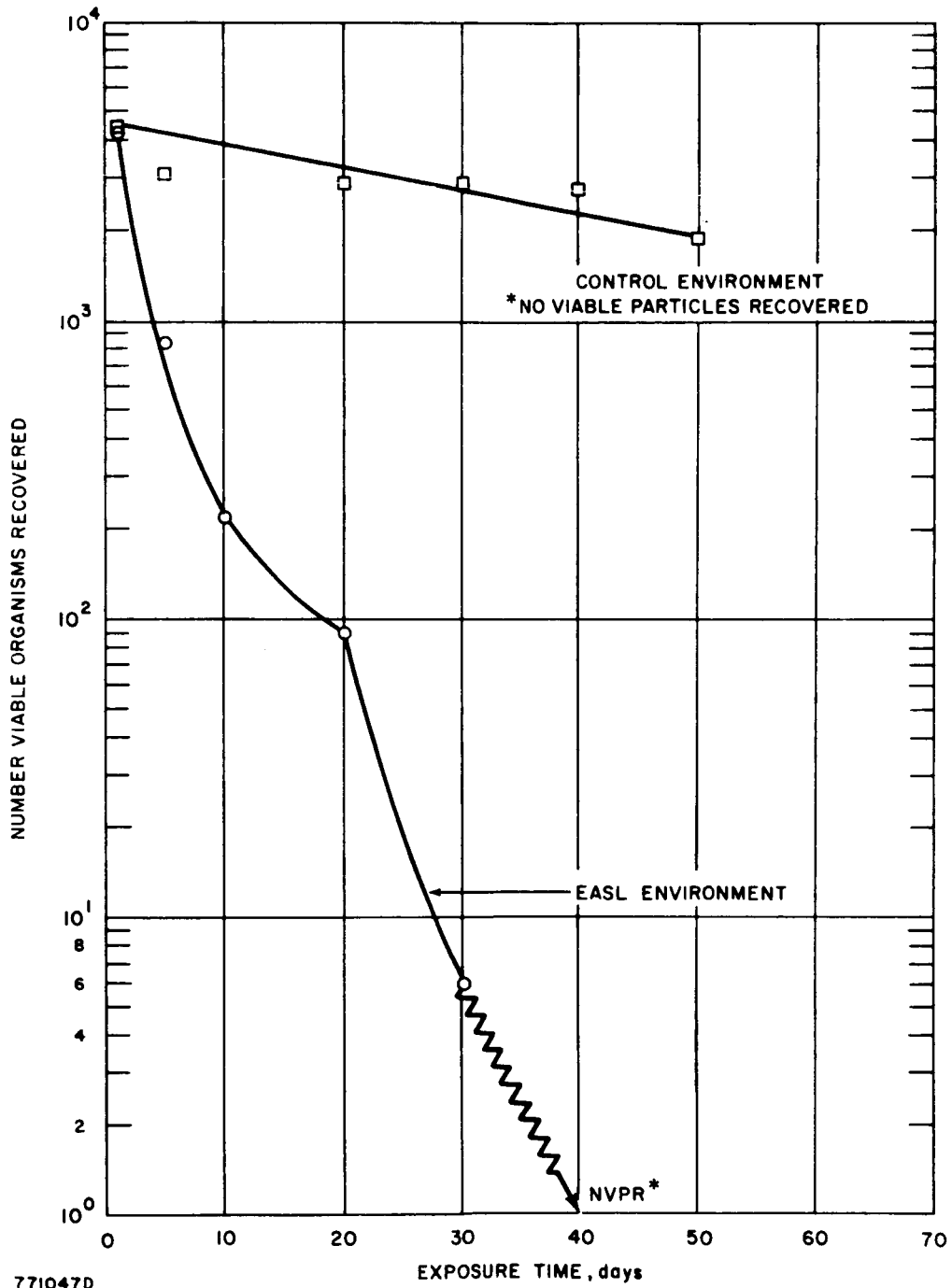


Figure 15. DIE-OFF OF EASL SPORE BACILLUS PUMILUS (M-2) ON CONVERSION COATED MAGNESIUM IN EASL LAMINAR FLOW AND IN THE CONTROL ENVIRONMENT

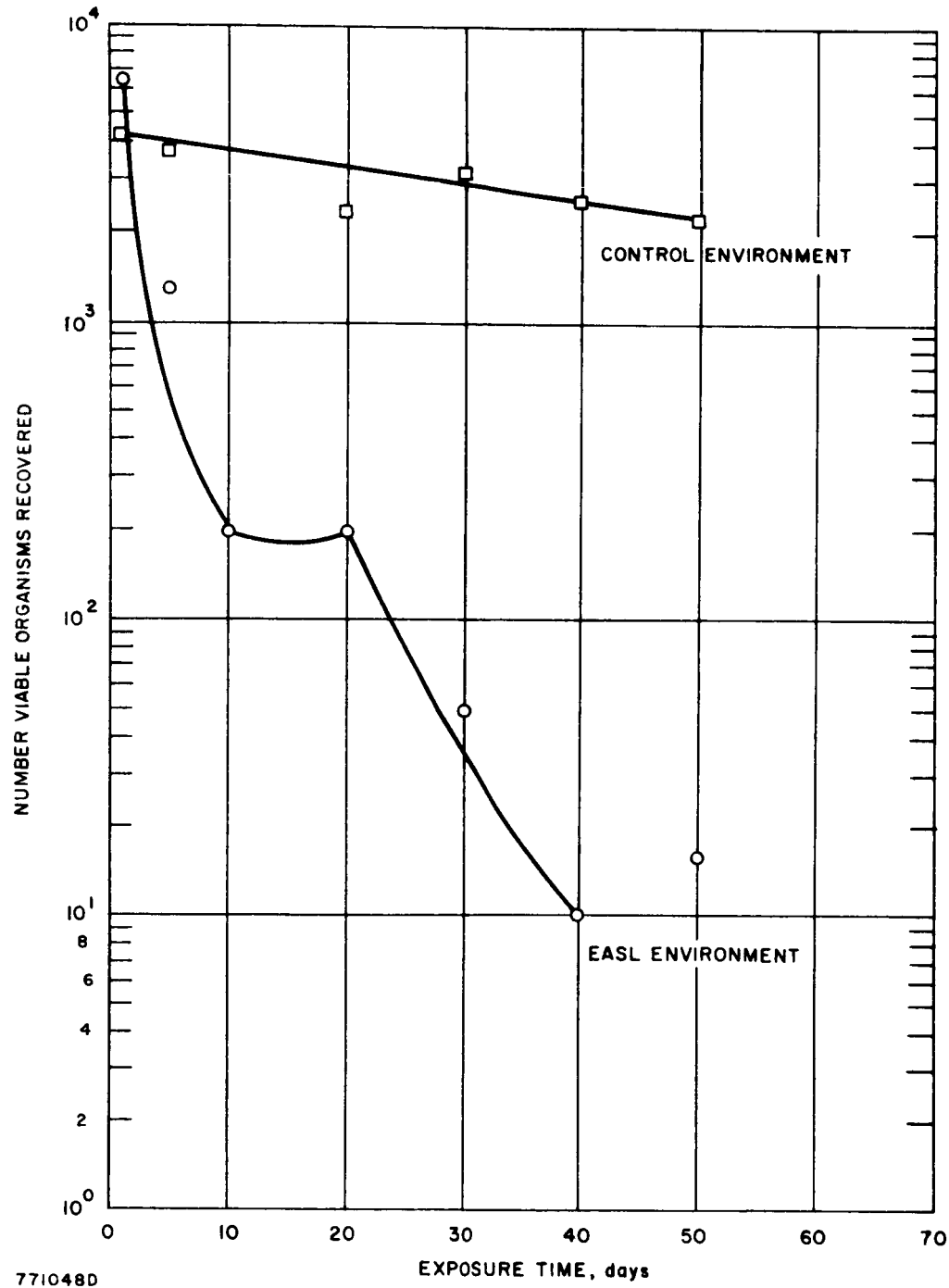


Figure 16. DIE-OFF OF EASL SPORE *BACILLUS PUMILUS* (M-2) ON CON-FORMAL COATING IN EASL LAMINAR FLOW AND IN THE CONTROL ENVIRONMENT

TABLE I  
DIE-OFF OF STAPHYLOCOCCUS EPIDERMIDIS WHEN  
EXPOSED TO THE EASL LAMINAR FLOW ENVIRONMENT

Stainless Steel Surface		Aluminum Surface	
Time	Surviving Organisms	Time	Surviving Organisms
0 hour	$1.3 \times 10^3$	0 hour	$3.35 \times 10^3$
6 hours	$4.9 \times 10^2$	6 hours	$1.3 \times 10^3$
24 hours	$2.0 \times 10^1$	24 hours	$1.1 \times 10^2$
48 hours	0	48 hours	$3.5 \times 10^1$
72 hours	0	72 hours	$0.2 \times 10^1$
5 days	0	5 days	0
10 days	0	10 days	$0.4 \times 10^1$
14 days	0	14 days	0

Conformal Coated Surface		Conversion-Coated Magnesium Surface (Dow 7)	
Time	Surviving Organisms	Time	Surviving Organisms
0 hour	$2.7 \times 10^3$	0 hour	$3.7 \times 10^3$
6 hours	$9.6 \times 10^2$	6 hours	$1.2 \times 10^3$
24 hours	$6.1 \times 10^2$	24 hours	$3.6 \times 10^2$
48 hours	$1.3 \times 10^2$	48 hours	$1.1 \times 10^2$
72 hours	$2.6 \times 10^1$	72 hours	$0.4 \times 10^1$
5 day	$1.6 \times 10^1$	5 day	0
10 day	0	10 day	$0.1 \times 10^1$
14 day	0	14 day	0

TABLE II  
DIE-OFF OF STAPHYLOCOCCUS EPIDERMIDIS WHEN  
EXPOSED TO THE CONTROL ENVIRONMENT

Stainless Steel Surface		Aluminum Surface	
Time	Surviving Organisms	Time	Surviving Organisms
0 hour	$1.35 \times 10^3$	0 hour	$3.35 \times 10^3$
6 hours	$1.0 \times 10^3$	6 hours	$2.0 \times 10^3$
24 hours	$4.3 \times 10^2$	24 hours	$9.5 \times 10^2$
48 hours	$2.7 \times 10^2$	48 hours	$5.4 \times 10^2$
72 hours	$2.3 \times 10^2$	72 hours	$4.5 \times 10^2$
5 days	$1.4 \times 10^1$	5 days	$9.3 \times 10^1$
10 days	$0.2 \times 10^1$	10 days	$2.6 \times 10^1$
14 days	$0.4 \times 10^1$	14 days	0

Conformal Coated Surface		Conversion-Coated Magnesium Surface (Dow 7)	
Time	Surviving Organisms	Time	Surviving Organisms
0 hour	$2.7 \times 10^3$	0 hour	$3.7 \times 10^3$
6 hours	$2.0 \times 10^3$	6 hours	$1.6 \times 10^3$
24 hours	$5.6 \times 10^2$	24 hours	$5.7 \times 10^2$
48 hours	$3.6 \times 10^2$	48 hours	$3.0 \times 10^2$
72 hours	$1.2 \times 10^2$	72 days	$1.6 \times 10^2$
5 days	$6.9 \times 10^1$	5 days	$4.9 \times 10^1$
10 days	0	10 days	$2.6 \times 10^1$
14 days	$0.1 \times 10^1$	14 days	$0.8 \times 10^1$



TABLE III  
DIE-OFF OF ESCHERICHIA COLI WHEN EXPOSED TO  
THE EASL LAMINAR FLOW ENVIRONMENT

Stainless Steel Surface		Aluminum Surface	
Time (hours)	Surviving Organisms	Time (hours)	Surviving Organisms
0	$8.3 \times 10^2$	0	$2.3 \times 10^2$
2	$6.4 \times 10^2$	2	$1.3 \times 10^2$
4	$4.2 \times 10^1$	4	$3.4 \times 10^1$
6	$1.4 \times 10^1$	6	$2.4 \times 10^1$
24	0	24	0
48	0	48	0

Conformal Coated Surface		Conversion-Coated Magnesium Surface (Dow 7)	
Time (hours)	Surviving Organisms	Time (hours)	Surviving Organisms
0	$8.3 \times 10^2$	0	$8.3 \times 10^2$
2	$1.0 \times 10^1$	2	$1.5 \times 10^2$
4	0	4	$5.2 \times 10^1$
6	$0.8 \times 10^1$	6	$2.4 \times 10^1$
24	0	24	0
48	0	48	$0.2 \times 10^1$

TABLE IV  
DIE-OFF OF ESCHERICHIA COLI WHEN EXPOSED  
TO THE CONTROL ENVIRONMENT

Stainless Steel Surface		Aluminum Surface	
Time (hours)	Surviving Organisms	Time (hours)	Surviving Organisms
0	$8.3 \times 10^2$	0	$2.3 \times 10^2$
2	0	2	$1.5 \times 10^2$
4	$4 \times 10^1$	4	$9.9 \times 10^1$
6	0	6	$5 \times 10^1$
24	0	24	0
48	$0.2 \times 10^1$	48	$0.2 \times 10^1$

Conformal Coated Surface		Conversion-Coated Magnesium Surface (Dow 7)	
Time (hours)	Surviving Organisms	Time (hours)	Surviving Organisms
0	$8.3 \times 10^2$	0	$8.3 \times 10^2$
2	$6.3 \times 10^2$	2	$1.57 \times 10^2$
4	$1.19 \times 10^2$	4	$4.8 \times 10^1$
6	$1.5 \times 10^2$	6	$4.8 \times 10^1$
24	0	24	$0.6 \times 10^1$
48	$0.3 \times 10^1$	48	0

TABLE V  
DIE-OFF OF BACILLUS GLOBIGII SPORES WHEN EXPOSED  
TO THE EASL LAMINAR FLOW ENVIRONMENT

Stainless Steel Surface		Aluminum Surface	
Time (days)	Surviving Organisms	Time (days)	Surviving Organisms
1	$1.5 \times 10^2$	1	$2.5 \times 10^2$
5	$8 \times 10^1$	5	$6 \times 10^1$
10	$7 \times 10^1$	10	$4 \times 10^1$
20	$6 \times 10^0$	20	0
30	0	30	0
40	0	40	0
50	0	50	$2 \times 10^0$

Conformal Coated Surface		Conversion-Coated Magnesium Surface (Dow 7)	
Time (days)	Surviving Organisms	Time (days)	Surviving Organisms
1	$3.2 \times 10^2$	1	$3.8 \times 10^2$
5	$1.4 \times 10^2$	5	$1.6 \times 10^2$
10	$7 \times 10^1$	10	$7 \times 10^1$
20	$8 \times 10^0$	20	$2 \times 10^0$
30	$4 \times 10^0$	30	0
40	$2 \times 10^0$	40	0
50	0	50	0

TABLE VI  
DIE-OFF OF BACILLUS GLOBIGII SPORES  
WHEN EXPOSED TO CONTROLLED ENVIRONMENT

Stainless Steel Surface		Aluminum Surface	
Time (days)	Surviving Organisms	Time (days)	Surviving Organisms
1	$4.4 \times 10^2$	1	$5.6 \times 10^2$
5	$3.5 \times 10^2$	5	$4.9 \times 10^2$
10	$4.4 \times 10^2$	10	$4.9 \times 10^2$
20	$1.8 \times 10^2$	20	$3.6 \times 10^2$
30	$2.3 \times 10^2$	30	$3.2 \times 10^2$
40	$1.8 \times 10^2$	40	$2.1 \times 10^2$
50	$1.2 \times 10^2$	50	$2.4 \times 10^2$

Conformal Coated Surface		Conversion-Coated Magnesium Surface (Dow 7)	
Time (days)	Surviving Organisms	Time (days)	Surviving Organisms
1	$4.4 \times 10^2$	1	$1.9 \times 10^2$
5	$3.9 \times 10^2$	5	$1.9 \times 10^2$
10	$3.1 \times 10^2$	10	$2.9 \times 10^2$
20	$1.7 \times 10^2$	20	$1.5 \times 10^2$
30	$2.3 \times 10^2$	30	$1.7 \times 10^2$
40	$1.6 \times 10^2$	40	$4 \times 10^1$
50	$1.3 \times 10^2$	50	$4 \times 10^1$

TABLE VII  
DIE-OFF OF BACILLUS PUMILUS (M-2) WHEN EXPOSED  
TO THE EASL LAMINAR FLOW  
ENVIRONMENT

Stainless Steel Surface		Aluminum Surface	
Time (days)	Surviving Organisms	Time (days)	Surviving Organisms
1	$4.5 \times 10^3$	1	$5.3 \times 10^3$
5	$1.2 \times 10^3$	5	$6.5 \times 10^2$
10	$1.1 \times 10^2$	10	$1.3 \times 10^2$
20	$1.2 \times 10^3$	20	$8 \times 10^0$
30	$0.2 \times 10^1$	30	$2 \times 10^0$
40	$1.6 \times 10^1$	40	0
50	$6 \times 10^0$	50	0

Conformal Coated Surface		Conversion-Coated Magnesium Surface (Dow 7)	
Time (days)	Surviving Organisms	Time (days)	Surviving Organisms
1	$6.6 \times 10^3$	1	$4.2 \times 10^3$
5	$1.3 \times 10^3$	5	$8.5 \times 10^2$
10	$2 \times 10^2$	10	$2.2 \times 10^2$
20	$2 \times 10^2$	20	$9 \times 10^1$
30	$5 \times 10^1$	30	$6 \times 10^0$
40	$1 \times 10^1$	40	0
50	$1.6 \times 10^1$	50	$4 \times 10^0$

TABLE VIII  
DIE-OFF OF BACILLUS PUMILUS (M-2) WHEN EXPOSED  
TO THE CONTROL ENVIRONMENT

Stainless Steel Surface		Aluminum Surface	
Time (days)	Surviving Organisms	Time (days)	Surviving Organisms
1	$4 \times 10^3$	1	$5.4 \times 10^3$
5	$4 \times 10^3$	5	$4.1 \times 10^3$
10	$0.3 \times 10^3$	10	$0.4 \times 10^3$
20	$2.7 \times 10^3$	20	$1.9 \times 10^3$
30	$2.5 \times 10^3$	30	$3.2 \times 10^3$
40	$1.5 \times 10^3$	40	$2.4 \times 10^3$
50	$1.4 \times 10^3$	50	$2.1 \times 10^3$

Conformal Coated Surface		Conversion-Coated Magnesium Surface (Dow 7)	
Time (days)	Surviving Organisms	Time (days)	Surviving Organisms
1	$4.3 \times 10^3$	1	$4.5 \times 10^3$
5	$3.8 \times 10^3$	5	$3.1 \times 10^3$
10	$0.5 \times 10^3$	10	$0.33 \times 10^3$
20	$2.4 \times 10^3$	20	$2.9 \times 10^3$
30	$3.2 \times 10^3$	30	$2.9 \times 10^3$
40	$2.6 \times 10^3$	40	$2.8 \times 10^3$
50	$2.3 \times 10^3$	50	$1.9 \times 10^3$

TABLE IX

DIE-OFF OF A MIXTURE OF EASL ISOLATES, ([B. PUMILUS]M-2),  
 ([ARTHROBACTER GLOBIFORMIS]E-17), ([MICROCOCCUS LUTEUS]E-6)  
 AND ([MICROCOCCUS RHODOCHROUS]E-9) EXPOSED TO THE EASL  
 ENVIRONMENT ON STEEL, ALUMINUM, CONVERSION, AND CONFORMAL-  
 COATED MAGNESIUM SURFACES

<u>Stainless Steel Surface</u> Number of Viable Organisms					<u>Aluminum Surface</u> Number of Viable Organisms				
Time (days)	E-17	E-9	E-6	Spore M-2	Time (days)	E-17	E-9	E-6	M-2
1	15	0	2	159	1	105	0	0	54
5	51	0	2	51	5	22	0	0	22
10	10	0	0	53	10	2	0	0	26
20	2	0	0	50	20	0	0	0	60
30	0	0	0	64	30	0	0	0	38
40	0	0	0	72	40	0	0	0	19
50	0	0	0	49	50	0	0	0	47

<u>Conformal Coated Surface</u> Number of Viable Organisms					<u>Conversion-Coated Magnesium Surface (Dow 7)</u> Number of Viable Organisms				
Time (days)	E-17	E-9	E-6	M-2	Time (days)	E-17	E-9	E-6	M-2
1	120	0	0	120	1	121	0	0	121
5	16	0	0	16	5	75	0	2	75
10	0	0	0	166	10	10	0	2	120
20	12	0	0	130	20	12	0	0	140
30	0	0	0	88	30	0	0	0	135
40	4	0	0	117	40	0	0	0	151
50	1	0	0	105	50	0	0	0	107

TABLE X  
DIE-OFF OF A MIXTURE OF EASL ISOLATES,  
(BACILLUS PUMILUS]M-2), (ARTHROBACTER GLOBIFORMIS]E-17),  
(MICROCOCCUS LUTEUS]E-6) AND (MICROCOCCUS RHODOCHROUS]E-9)  
EXPOSED TO THE CONTROL ENVIRONMENT ON STEEL, ALUMINUM,  
CONVERSION-COATED MAGNESIUM AND CONFORMAL-  
COATED SURFACES

<u>Stainless Steel Surfaces</u> Number of Viable Organisms					<u>Aluminum Surfaces</u> Number of Viable Organisms				
Time (days)	E-17	E-9	E-6	M-2	Time (days)	E-17	E-9	E-6	M-2
1	25	0	0	155	1	92	2	1	143
5	315	0	0	182	5	9	0	2	105
10	13	0	2	303	10	49	0	6	308
20	8	0	4	90	20	8	0	2	135
30	10	0	0	87	30	17	0	0	123
40	8	0	0	115	40	5	0	12	86
50	5	0	0	107	50	5	0	0	46

<u>Conformal Coated Surface</u> Number of Viable Organisms					<u>Conversion-Coated Magnesium Surface (Dow 7)</u> Number of Viable Organisms				
Time (days)	E-17	E-9	E-6	M-2	Time (days)	E-17	E-9	E-6	M-2
1	101	1	1	233	1	157	0	0	169
5	1,652	0	6	217	5	99	0	2	196
10	13	0	2	262	10	29	0	0	349
20	23	0	0	71	20	13	1	0	146
30	4	0	0	91	30	2	0	0	155
40	0	0	0	152	40	9	0	7	153
50	0	0	0	136	50	0	0	0	99



TABLE XI

THE ACCUMULATION OF BIOLOGICAL BURDEN ON FALLOUT STRIPS  
OF STAINLESS STEEL, ALUMINUM, CONFORMAL-COATED  
MATERIAL, AND CONVERSION-COATED MAGNESIUM  
EXPOSED TO THE EASL ENVIRONMENT

Time	Stainless Steel Surface (3 in <sup>2</sup> ) Replicates of 5			Aluminum Surface (3 in <sup>2</sup> ) Replicates of 5			Conformal Coated Material Surface (3 in <sup>2</sup> ) Replicates of 5			Conversion Coated Magnesium Surface (3 in <sup>2</sup> ) Replicates of 5		
	Range		Average Per Assay	Range		Average Per Assay	Range		Average Per Assay	Range		Average Per Assay
	High	Low		High	Low		High	Low		High	Low	
0 hour	0	0	0	0	0	0	0	0	0	0	0	0
6 hour	7	0	2	50	0	16	10	0	5	30	0	8
1 day	10	0	2	2	0	1	30	0	12	20	0	8
2 days	20	0	12	20	0	8	20	0	4	0	0	0
3 days	0	0	0	10	0	4	20	0	11	20	0	13
5 days	100	0	54	100	0	38	150	0	38	100	0	62
10 days	0	0	0	50	0	25	57	1	21	32	0	14
20 days			22	126	2	60	50	0	25	24	7	11
30 days	150	0	40	100	0	52	150	0	30	0	0	0
40 days	200	50	101	200	50	11	100	0	27	100	0	26
50 days	5	0	2	50	0	25	1	0	1	150	0	82

TABLE XII  
EASL ENVIRONMENT, TEMPERATURE, AND HUMIDITY DURING  
THE COURSE OF TASK 5.3 CONTAMINATION PLATEAU  
STUDY

Date				Humidity	Temperature
Jan	9	Day	1 (Start of study)	*	*
	10		2	*	*
	11		3	*	*
	12		4	*	*
	13		5	*	*
	14		6	*	*
	15		7	*	*
	16		8	*	*
	17		9	*	*
	18		10	*	*
	19		11	*	*
	20		12	*	*
	21		13	*	*
	22		14	*	*
	23		15	*	*
	24		16	*	*
	25		17	*	*
	26		18	*	*
	27		19	*	*
	28		20	*	*
	29		21	*	*
	30		22	*	*
	31		23	*	*
Feb	1		24	*	*
	2		25	33 percent RH	*
	3		26	27 percent RH	82°F
	4		27	27 percent RH	82°F
	5		28	27 percent RH	82°F
	6		29	27 percent RH	82°F
	7		30	39 percent RH	*
	8		31	*	*
	9		32	*	*
	10		33	*	*
	11		34	*	*
	12		35	*	*
	13		36	*	*

\*Met Specification (Temperature 70°F ± 10; Humidity 45-50-percent RH.

TABLE XII (Concl'd)

Date				Humidity	Temperature
Feb	14	Day	37	*	*
	15		38	*	*
	16		39	*	*
	17		40	*	*
	18		41	*	*
	19		42	*	*
	20		43	37 percent RH	*
	21		44	37-38 percent RH	*
	22		45	36-39 percent RH	*
	23		46	33-37 percent RH	*
	24		47	36 percent RH	*
	25		48	36 percent RH	*
	26		49	36 percent RH	*
	27		50	31-37 percent RH	*
	28		51 (End of study)	37-39 percent RH	*

\*Met Specification (Temperature 70°F ± 10; Humidity 45-50 -percent RH.

**TABLE XIII**  
**DIE-OFF OF MICROORGANISMS EXPOSED TO THE EASL LAMINAR**  
**FLOW ENVIRONMENT**

Exposure Time	<u>E. coli</u> Material				<u>S. epidermidis</u> Material				Mixture of EASL Isolates Material				<u>B. globigii</u> Spores Material				<u>B. pumilus</u> spores (M-2) Material			
	S.S.	Al	C.C.	Con	S.S.	Al	C.C.	Con	S.S.	Al	C.C.	Con	S.S.	Al	C.C.	Con	S.S.	Al	C.C.	Con
0 hour	830	230	830	830	1300	3350	2700	3700					400	400	400	400				
2 hours	640	130	10	150																
4 hours	420	340	0	52																
6 hours	140	240	8	24	490	1300	960	1200												
1 day	0	0	0	0	20	110	610	360	176	159	240	242	150	250	320	380	4500	5300	6600	4200
2 days	0	0	0	2	0	35	130	110												
3 days					0	2	26	4												
5 days					0	0	16	0	104	44	32	152	80	60	140	160	1200	650	1300	850
10 days					0	4	0	1	63	28	166	132	70	40	70	70	110	130	200	220
14 days					0	0	0	0												
20 days									52	60	142	152	6	0	8	2	112	8	200	90
30 days									64	38	88	135	0	0	4	0	20	2	50	6
40 days									72	19	121	151	0	0	2	0	16	0	10	0
50 days									49	47	106	107	0	2	0	0	6	0	16	4

S.S. = Stainless Steel

Al = Aluminum

C.C. = Conformal Coating

Con Mg = Conversion Coated Magnesium (Dow 7)

TABLE XIV

Exposure Time	E. coli Material				S. epidermidis Material				Mixture of EASL Isolates Material				B. globigii Spores Material				B. pumilus Spores (M-2) Material			
	S.S. A1		C. C.	Con Mg	S.S. A1		C. C.	Con Mg	S.S. A1		C. C.	Con Mg	S.S. A1		C. C.	Con Mg	S.S. A1		C. C.	
0 hour	830	230	830	830	1300	3350	2700	3700						400	400	400	4100	5300	4350	
2 hours	0	150	630	157																
4 hours	40	99	119	48																
6 hours	0	50	150	48	1000	2000	2000	1600												
1 day	0	0	0	6	432	950	560	570	180	238	336	326	440	560	440	190	5400	4300	4500	
2 days	20	2	3	0	270	540	360	300												
3 days					230	450	120	160												
5 days					14	93	69	49	497	116	1875	297	350	490	390	190	4100	3800	3100	
10 days					2	26	0	26	318	363	277	378	440	490	310	290	400	500	330	
14 days					4	0	1	8												
20 days									102	145	94	160	180	360	170	150	1900	2400	2900	
30 days									97	140	95	157	230	320	230	170	3200	3200	2900	
40 days									123	103	152	169	180	210	160	40	2400	2600	2800	
50 days									112	51	136	99	120	240	130	60	2100	2300	1900	

S.S. = Stainless Steel  
Al = Aluminum  
C.C. = Conformal Coating  
Con Mg = Conversion Coating

IV. DISCUSSION

It was seen from the experimental results that exposure of bacteria (heterotrophic mesophiles), both vegetative cells and spores, to the EASL laminar flow environment significantly enhances their kill when compared to the die-off rate in a controlled environment. (See Figures 1-12.) An exception to this situation can be seen in Figure 5 where E. coli may have died more rapidly in the control environment than in the laminar flow environment. Since the numbers of viable organisms recovered at the 6-hour sampling time was so low ( $1.4 \times 10^1$  in the EASL environment versus 0 in the control environment), it was felt that the difference in die-off rates was not significant.

The type of surface on which the organisms were exposed to the EASL environment appears to play a role in the organism's die-off. When examining the die-off curves of the individual organisms on the four types of surfaces (stainless steel, aluminum, conversion-coated magnesium, and conformal-coated material) variations of die-off rates were observed. The time of maximum kill was delayed on the conversion-coated magnesium and conformal-coated surfaces. This phenomenon is not as evident with the vegetative cells which are more sensitive to the lethal effects of adverse conditions than are spores. A similar protective effect was noted in the Germicide Study (Task 5.1) when organisms on epoxy painted surfaces appeared more resistant to the kill of the germicides than the same organism on a stainless steel or aluminum surface. This protective effect might be due to surface finish: smoothness, stickiness, charge, or a residual chemical contaminant. On the basis of present information, this phenomenon can only be described, not explained. Further experimentation is required to ascertain if a protective effect does truly exist and what the possible mechanism or mechanisms could be.

The die-off EASL isolates generally followed that obtained with individual species of organisms. That is, exposure to the EASL environment laminar flow was seen to accelerate die-off when compared to the controlled environment. In the EASL mix, the vegetative cells did not appear after 10 days except for A. globiformis. This organism forms cyst-like structures which appear to resist desiccation in a manner similar to the bacillus spores.

There was a difference between die-off of organisms when exposed to the EASL and controlled environments individually or in mixtures. The die-off individual organisms in mixtures with other organisms was slower than individual organisms exposed one at a time. Here again, a possible protective effect, in this case due to bacterial carcasses (denatured proteins and other compounds of the bacterial cell residue) might be the answer. In this situation one can only postulate possible mechanisms and suggest further investigation for verification and explication of the phenomenon.

The curves for the spores exposed to the laminar down flow appear to have two distinct slopes in some cases (Figures 9-13, 15 and 16). This difference in rate of kill may reflect the presence of vegetative cells in the spore population or it may represent differences in resistance to death by desiccation within the spore population itself.

It can be seen from some of the curves and tables, that the individual data points do not always correlate, but appear to be even random (Figure 11). The following factors may account for these apparent inconsistencies.

1. Uneven inoculation of the coupons.
2. Variations in the removal of the organisms from the coupons during sonication.
3. Dispersal of cell aggregates following sonication and differential drying of layers of the inoculum resulting in changes in cell resistance to drying.
4. Protection of living cells by debris from dead cells and residual nutrient.
5. An interaction between the liquid inoculum.
6. The surface coating on the coupon, especially the conversion-coated material.

In addition, contamination prior to or during the assay procedure must not be totally eliminated as contributing to occasionally aberrant results.

A variable that unavoidably occurred during this study was the failure of the EASL facility to retain specified temperature and humidity during two periods of this study-- February 2-7, 1967 and February 20-28, 1967, (see Table XII.) The exact impact resulting from these two failures of EASL to meet specifications is not known, but it is assumed to be a limited one. The EASL specification for humidity is 45-percent RH  $\pm$  5-percent and for temperature is 70° F  $\pm$  10° F. The lowest humidity recorded was 27 percent and the highest temperature was 80° F. The EASL facility did not exceed the high specification for humidity or the low specification for temperature.

All curves were drawn in such a manner that the first zero point was considered the end point of viability for the organisms assayed. This was done for the purposes of consistency and due to the fact that any subsequent variance from the end point was felt not to be significant.

## V. CONCLUSIONS

- a. Vertical laminar flow will accelerate the die-off of microorganisms (heterotrophic mesophilic bacteria, vegetative cells with spores).
- b. Die-off of the same species of microorganism exposed to vertical laminar flow on different types of surfaces varies. This is a tentative conclusion and requires further investigation.

- c. Die-off of microorganisms varies in mixtures (Two or more species or genes) rather than as individuals when exposed to vertical laminar flow. This is a tentative conclusion and requires further investigation.
- d. Vegetative cells die more rapidly than spores when exposed to vertical laminar flow.



## VI. RECOMMENDATIONS

- a. Vertical laminar flow could be used to reduce the biological burden on components, subassemblies, and structures with exposed surface burden.
- b. Further study should be made of the role played by surfaces on which microorganisms are exposed to laminar flow.
- c. Further investigation should be done to determine the effects of vertical laminar flow kill on individual species of microorganisms versus mixtures of species of microorganisms.

## VII. REFERENCES

1. McDade, J., et al., Environmental Microbiology and the Control of Microbial Contamination, cited from Spacecraft Sterilization Technology, NASA SP-108, Pasadena, California, November 16-18, 1965.